

## Differentiation of *Ohomopterus yaconinus* and its “Subspecies” (Coleoptera, Carabidae) Inferred from the DNA Sequences of Mitochondrial ND5 Gene and Internal Transcribed Spacer I (ITS I)

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**Abstract** The phylogenetic properties of *Ohomopterus yaconinus* collected from most of its distributional range have been examined by sequence comparisons of the mitochondrial ND5 gene and the nuclear ITS I. In the ND5 phylogenetic tree are recognized two clades: the western clade is monophyletic without contamination of any other species; the other one, the Kinki clade, is intermingled with several other species including *O. iwawakianus*. The ND5 gene of *O. yaconinus* in the Kinki District is supposed to be replaced by that of *O. iwawakianus* when the western population invaded the Kinki District. The ITS I of the Kinki population is clustered either with that of the western population of *O. yaconinus* or with that of *O. iwawakianus*. These results together with the distribution profiles of these two taxa suggest strongly that the western population of *O. yaconinus* would be the authentic strain of this species, while that in the Kinki District is the offspring of hybrids between the authentic *O. yaconinus* and *O. iwawakianus*. *Ohomopterus yaconinus* from the Noto Peninsula is considered to be the hybrid-derivative formed between the male of authentic *O. yaconinus* and the female of *O. arrowianus*.

### Introduction

*Ohomopterus yaconinus*<sup>\*\*)</sup> is a well-known carabid beetle endemic to western Japan, extending its distribution over such districts as Chûgoku, Shikoku, Kinki and a part of Hokuriku and Chûbu. Morphologically, this species is characterized mainly by

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<sup>\*\*) For the higher classification of the subtribe Carabina, we follow the system proposed by IMURA (2002) and OSAWA *et al.* (2004).</sup>

pentagonally shaped copulatory piece of the male genital organ. It was originally described by BATES (1873, p. 231) as a distinct species of the genus *Carabus* (s. lat.) from “Nagasaki and Hiogo (=Hyôgo of Kôbe in Hyôgo Prefecture)”, though the former locality is most probably incorrect. In 1934, BREUNING described a new species under the name of *Apotomopterus Blaieri* from “Fushiki” (Toyama Prefecture). In his early works on the Japanese Carabina, NAKANE (1953) regarded these two taxa as belonging to a single species (=*Apotomopterus yaconinus* in his sense), and divided it into four subspecies, namely, nominotypical *yaconinus*, *blairi*, *iwawakianus* and *kiensis*. Of these, the latter two were described at that time and are now regarded as different species (see IMURA *et al.*, 2005, on other pages of this volume). Since then, except for a brief discussion by KOMIYA (1971), further taxonomical study on this species has been left almost untouched for more than 40 years, presumably because of its seemingly poor local variation and continuous distribution. In 1994, ISHIKAWA and KUBOTA made a tentative revision of the species (=*Carabus (Ohomopterus) yaconinus* in their sense) based on the morphology, and recognized in it totally eight subspecies, six of which were newly described at that time. The names of these subspecies and their distributional ranges are as follows:

- 1) subsp. *blairi* (Toyama and Ishikawa Prefs.).
- 2) subsp. *sotai* (eastern Kyoto, Shiga, northern Mie and southwestern Gifu Prefs.).
- 3) subsp. *cupidicornis* (central Mie, northern Nara, Osaka and northern Wakayama Prefs.).
- 4) subsp. *yaconinus* (Hyôgo Pref. including Is. Awaji-shima, western Kyoto, southern Okayama and Hiroshima Prefs.).
- 5) subsp. *oki* (Is. Dôgo of the Oki Islands in Shimane Pref.).
- 6) subsp. *maetai* (Tottori, Shimane and northern Hiroshima Prefs.).
- 7) subsp. *yamaokai* (northern part of Is. Shikoku including Iss. Naka-jima and Gogo-shima, and northwestern Wakayama Pref.).
- 8) subsp. *seto* (Iss. Nuwa-shima and Futagami-jima in Ehime Pref.).

Two years later, SU *et al.* (1996) constructed a phylogenetic tree of the representative species of the genus *Ohomopterus* in Japan using mitochondrial ND5 gene sequences, and showed that *O. yaconinus* fell out in two different places on the tree. One group, which they called WJP (the western Japan lineage; called **W-yaconinus** in this paper) is exclusively composed of *O. yaconinus* without contamination of any other *Ohomopterus* species, suggesting that *W-yaconinus* is most probably monophyletic. The other one, occurring together with a number of other *Ohomopterus* species, formed a cluster called KNK (the Kinki lineage; called **K-yaconinus** in this paper). Since the evolutionary distance of the ND5 gene between these two “populations” is considerably far, they must have the evolutionary histories different from each other.

In the present paper, we have analyzed 26 specimens of *O. yaconinus* including the previously reported ones (SU *et al.*, 1996) from various localities which cover almost entire distributional ranges of the species, for the sequences of the ND5 gene and

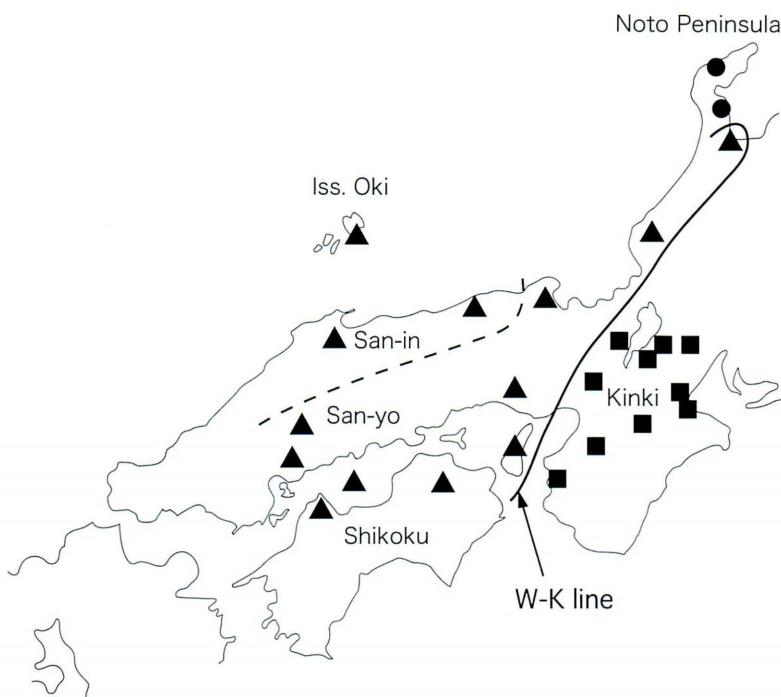


Fig. 1. Map showing the localities of the specimens of *Ohomopterus yaconinus* analyzed in this study, on which the distributional boundary of W- and K-yaconinus (W-K line) is shown. —▲, W-yaconinus; ■, K-yaconinus; ●: *O. yaconinus* with the ND5 gene sequence of the *O. arrowianus*-type.

nuclear ITS I to know more detailed phylogeny and distributional profiles of this species.

## Materials and Methods

Figure 1 shows the localities of the samples analyzed in this study. The analytical methods are the same as those described by SU *et al.* (1996) for the ND5 gene and by TOMINAGA *et al.* (2005) for the ITS I. The accession numbers for DDBJ, EMBL and GenBank of the specimens used in this study will be shown collectively in the last paper of this series on the Japanese *Ohomopterus* species.

## Results

### Phylogenetic tree of the ND5 gene

*Ohomopterus yaconinus* appeared in two different clusters in the ND5 phylogenetic tree of the *Ohomopterus* species, each containing W-yaconinus and K-yaconinus as defined above.

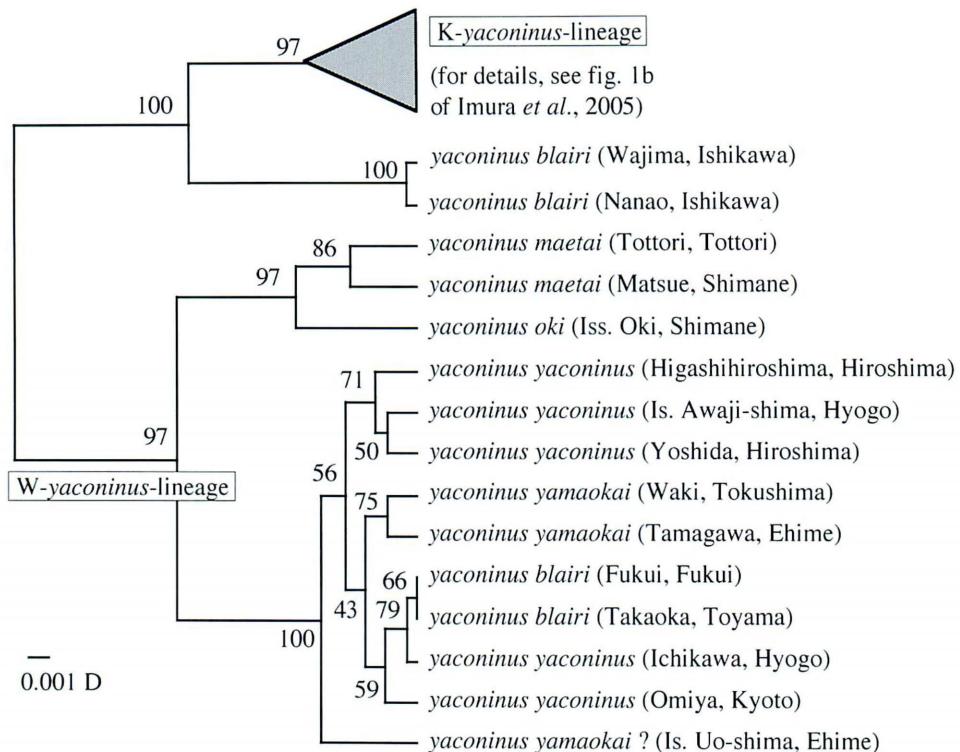


Fig. 2. Phylogenetic tree of ND5 gene from *Ohomopterus yaconinus* with special reference to W-yaconinus. For the tree of K-yaconinus, see IMURA *et al.* (2005, fig. 1-b). Two examples of *O. yaconinus* from Wajima and Nanao on the Noto Peninsula have the ND5 gene sequence of the *O. arrowianus* type.

The first one (referable to the W-yaconinus lineage; see Fig. 2) included the specimens from such districts as Chūgoku, Shikoku, northwestern Kinki and Hokuriku, and was further divided into two subclusters named the San-in and San-yō subclusters. The former contained the specimens collected from Tottori and Shimane (subsp. *maetai*), and the Islands of Oki (subsp. *oki*). The latter included the specimens from northern Shikoku (subsp. *yamaokai*), those from Hiroshima, Hyōgo and Kyoto Prefectures (subsp. *yaconinus*) and those from Fukui and Toyama Prefectures (subsp. *blairi*). In the San-in subcluster, the specimens referable to subsp. *maetai* and those referable to subsp. *oki* were well separated, while no significant difference was recognized among the three subspecies in the San-yō subcluster.

The second one (referable to the K-yaconinus lineage; see fig. 1b of IMURA *et al.*, 2005) included subspp. *sotai*, *cupidicornis* and *yamaokai* which did not form a monophyletic clade but randomly appeared together with the other species such as *O. arrowianus*, *O. maiyasanus*, *O. iwawakianus* and *O. dehaanii*.

So far as the analyzed specimens are concerned, the distributional boundary be-

tween W-*yaconinus* and K-*yaconinus* lies on the western margin of the Yodogawa-Biwa Lake line (hereafter called the **W-K line**; see Fig. 1).

Two specimens from the Noto Peninsula (Wajima and Nanao) corresponding to Nos. 20 and 21 in fig. 1 of TOMINAGA *et al.* (2005) carried the ND5 gene sequence of the *O. arrowianus*-type.

#### *Phylogenetic tree of the ITS I*

In the tree of the ITS I, the specimens of W-*yaconinus* and four individuals of K-*yaconinus* (from Osaka, Nara, Mie and Shiga Prefectures) formed a single cluster (see Cluster F in fig. 2 a of IMURA *et al.*, 2005) without contamination of any other *Ohomopterus* species. The examples of subsp. *blairi* from the Noto Peninsula were also included in the same cluster.

The other four specimens of K-*yaconinus* (from Nara and Mie Prefectures) were found in the other cluster (see Cluster A in fig. 2 b of IMURA *et al.*, 2005) together with some other species such as *O. arrowianus*, *O. iwawakianus*, *O. maiyasanus* and *O. uenoi*.

### Discussion

The sequence difference of the ND5 gene are sufficient enough to deduce not only the grouping of the specimens analyzed but also the branching order in the phylogenetic tree in most cases. On the contrary, the sequence differences of ITS I are small so that it can be used only for the grouping of the specimens and not for the determination of the branching order within each group. However, the ITS I gives us a useful information as to the biological properties, such as the relationship between the morphological classification and the origin of the “subspecies”.

#### *Phylogenetic properties of O. yaconinus in various regions*

In both the phylogenetic trees of the ND5 gene and that of the ITS I, W-*yaconinus* forms a single cluster without contamination of any other species. Such a congruence between the ND5 gene and the ITS I suggests that W-*yaconinus* is monophyletic without occurrence of hybridization with other species since the establishment of *O. yaconinus* as a species. In other words, W-*yaconinus* would most probably be the authentic species strain.

The ND5 of all the K-*yaconinus* individuals belongs to the Cluster III (see fig. 1 b of IMURA *et al.*, 2005), in which the ND5 from other species including *O. iwawakianus* are intermingled.

The ITS I of four individuals of K-*yaconinus* cannot be differentiated from that of W-*yaconinus*, which is of the Cluster F-type, in spite of the phylogenetic independence of the ND5 gene between them. The other three individuals of K-*yaconinus* carry the ITS I of the Cluster A-type and was clustered together with *O. iwawakianus*. There are

at least two possibilities to account for the occurrence of ITS I of *K-yaconinus* in these two different clusters. One would be that *K-yaconinus* having the F-type ITS I is a hybrid-derivative between the male of *K-yaconinus* and the female of *O. iwawakianus*. The individuals having the A-type ITS I would be the secondary hybrids between the female of *K-yaconinus* (a hybrid between the female of *W-yaconinus* and the male of *O. iwawakianus*) and the male of *O. iwawakianus*. Another possibility would be that all the individuals having either the F- or A-type are the secondary hybrids as mentioned above. The reason for the presence of the A-type, and not the F-type, of ITS I in some *K-yaconinus* would be that if the ITS I and the gene(s) responsible for the species-specific morphologies are separately exist on the same chromosome or on the different chromosomes, the inter- or intra-chromosomal chimera formation upon, or some generations after, hybridization would have occurred. There would be a chance for independent inheritance of the ITS I and the genes determining the morphological characters. It is then possible that the latter gene(s) was introduced to the hybrids independently from the ITS I at a certain stage during the successive hybridizations. In the individuals having the F-type ITS I, the genes for morphological characters and ITS I were introduced together into the secondary hybrids. At present, however, there is no evidence as to decide which possibility is correct. In either case, the results suggest that *K-yaconinus* is a hybrid-descendant between the male of *W-yaconinus* and *O. iwawakianus*. TOMINAGA (1999; see also OSAWA *et al.*, 2004) once proposed a scenario for the formation of *K-yaconinus* as follows: *W-yaconinus* would have invaded the Kinki District from the west and hybridized with *O. maiyasanus*. However, a close examination of the present phylogenetic trees, which include much more samples than in the previous one (SU *et al.*, 1996), suggests that *K-yaconinus* was resulted from the hybridization between *W-yaconinus* and *O. iwawakianus* (not *O. maiyasanus*) once or twice, as mentioned above. This new assumption may be supported by the fact that the range of *K-yaconinus* seems to be separated from that of *W-yaconinus* by the W-K line (see Fig. 1), whereas the distributional range of *O. iwawakianus* is rather restricted in the central and southern parts of the Kinki District, not extending westwards across this line. On the other hand, *O. maiyasanus* is distributed much more widely from the Hokuriku District to the northeastern part of the Chūgoku District along the coastal area of the Sea of Japan, where only *W-yaconinus* inhabits. Incidentally, it should be mentioned that *O. iwawakianus* is considered to be a hybrid-descendant between *O. maiyasanus* and “*kiensis*” (not a subspecies of *O. iwawakianus* but should be regarded as an independent species; see IMURA *et al.*, 2005), and therefore *K-yaconinus* would have resulted by the crossing between *W-yaconinus* (=authentic strain) and a hybrid-descendant between *O. maiyasanus* and “*kiensis*”.

The specimens from the Noto Peninsula (Wajima and Nanao) having *O. arrownianus*-type ND5 gene and *O. yaconinus*-type ITS I are also an example of the existence of a hybrid-derived population. Though we have tentatively regarded them as subsp. *blairi* in this paper, they reveal certain morphological differences from “authentic” *blairi*. They would have been formed in such a way that *K-yaconinus* in the San-

yô subcluster migrated towards northeast along the coastal area of the Sea of Japan, and the male of a part of them hybridized with the female of *O. arrowianus* somewhere on the Noto Peninsula in the past time.

As has been described in Introduction, the cluster containing W-*yaconinus* is further divided into the San-in and the San-yô subclades. Although subsp. *oki* is separated from subsp. *maetai* in the San-in subclade, the separation of the three subspecies in the San-yô subclade (*yamaokai*, *yaconinus* and *blairi*) cannot be possible on the phylogenetic tree. K-*yaconinus* contains two subspecies, *sotai* and *cupidicornis*, both of which are considered to be hybrid-descendants between the authentic *O. yaconinus* and *O. iwawakianus*. The problem is that the “subspecies” of *O. yaconinus* hitherto defined by the morphology alone are the mixture of those currently regarded as geographical races and offspring of the hybrids. It is obvious that these two should be discriminated from each other as two different categories, and cannot be summarized them into a single term such as “subspecies”. The geographical race must originate from the authentic ancestor of the species, whereas hybrid-derived individuals are more or less the chimeras of genetic characters derived from two or more species, even though they superficially resemble the authentic species.

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### 要 約

岡本宗裕・富永 修・蘇 智慧・井村有希・柏井伸夫・小鹿 亨・秋田勝己・大澤省三：ミトコンドリア ND5 遺伝子と核 ITS I DNA の分析に基づくヤコンオサムシとその「亜種」の分化について。——各地から集めたヤコンオサムシのミトコンドリア ND5 遺伝子と核 ITS I DNA を分析して分子系統樹を作成し、検討をくわえた。その結果、本種は、四国北部から中国地方（隠岐を含む）、さらに近畿北西部を経て北陸地方に至る範囲に分布する集団（西日本系ヤコン）と近畿地方を中心に分布する集団（近畿系ヤコン）の2系統に大きく分けられ、両者の境界（W-K line）は琵琶湖-淀川線のやや西方にあるらしいことが判明した。各系統樹の詳細な分析結果から、西日本系ヤコンは純系の独立種とみなすべきものだが、近畿系ヤコンはおそらく、イワワキオサムシ（=キイオサムシとマヤサンオサムシとの交雑集団）との交雑に由来する集団で、また、能登半島の集団は西日本系ヤコンとミカワオサムシとの交雑に由来するものであるらしいことが判明した。このように、従来、形態学からの所見のみに基づいて規定されてきた「亜種」のなかには、純系種内における地理的変異集団と、他種との交雑過程を経て形成さ

れた雑種由来の集団とが混在していることがあきらかになり、すべてを同一のカテゴリーに含めて論じてよいものかどうかという、きわめて興味深い問題が提起された。

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